REMARKS

1. Claim Rejections under 35 U.S.C. § 102

Claims 31-32, 34, 36, and 43 stand rejected under 35 U.S.C. § 102 as anticipated by Quarto et al. For the following reasons, this rejection should be withdrawn.

First, Quarto et al. does not disclose a method to identify cells having chondrocyte (phenotypic) stability. The Examiner asserts that Quarto et al. teach a method for determining the expression of positive and negative markers of chondrocyte (phenotypic) stability. Applicants submit that even if Quarto had provided 'a method to determine expression of positive and negative markers', which is contested, this in itself is not the same as providing a method to identify cells having chondrocyte (phenotypic) stability, as presently claimed. Indeed, Quarto et al. make no statement about the fact that the expressed collagen proteins are markers or should be considered as indicative of production of cartilage *in vivo*. At most, Quarto et al. use the expression of collagen proteins to identify the chondrocyte differentiation pathway of the cells, but there is no indication or suggestion that these proteins are or can be used to identify the chondrocyte (phenotypic) stability of the cells, i.e. the ability of cells to make stable cartilage. To the contrary, as detailed below, based on the results described in Quarto et al., such an interpretation would be incorrect. Thus, as such, the anticipation rejection is inappropriate.

In this regard it is noted that the Examiner has indicated that Quarto

describes a method for identifying the expression of a negative marker for chondrocyte (phenotypic) stability, thereby including claim 32 in the anticipation rejection. However, Quarto et al do not describe any negative markers for differentiation, let alone negative markers for chondrocyte (phenotypic) stability, i.e. markers, the *absence* of which is indicative of chondrocyte (phenotypic) stability.

The allegation by the Examiner that Quarto et al. have considered type X collagen as a negative marker is incorrect. Quarto et al. indicate that type X collagen is a specific marker of the hypertrophic stage (page 4966, right column, lines24-25). Quarto et al. thus, at most, consider this a positive marker for differentiation (page 4968, right column, second full paragraph, lines 11-14):

> *Differentiation in suspension culture was evidenced by the passage from type I to type II collagen synthesis and the accumulation of type X collagen, as specific marker of the hypertrophic stage.*

Thus, applicants submit that Quarto et al. does in no way consider the use of negative markers, let alone negative markers indicative of chondrocyte (phenotypic) stability. Again, in this regard, the anticipation rejection is inappropriate.

Applicants moreover submit that the Examiner's assessment of the contribution of Quarto et al. is incorrect. Quarto et al. do not teach a method to determine the expression of positive and negative markers *of chondrocyte (phenotypic) stability.*

The feature of "chondrocyte (phenotypic) stability", as indicated in the application refers to the capacity of a cell population to produce cartilage when injected *in vivo* without signs of vascular invasion or endochondral bone formation.

Applicants note that Quarto et al. did not observe this characteristic for any of the cell populations considered. Quarto et al. investigate the effect of different factors on the expression of collagen proteins and on the formation of cartilage *in vivo*. In all conditions of cell expansion and implantation tested, at least one vascularized tissue was formed together with cartilage by the implanted cells (see, for example, Figure 6 and Table 1).

This fact that Quarto et al. does not observe the formation of stable hyaline cartilage is important, and emphasizes the contribution of the present invention. Indeed, the present invention relates to identifying cells suitable for implantation in the treatment of a cartilage defect, based on their ability to produce stable hyaline cartilage *in vivo*. Applicants note that the formation by a cell population of cartilage tissue contaminated with bone or fat is undesirable, since these tissues do not contribute to cartilage repair. Accordingly, with regard to the alleged "markers" described by Quarto et al., as no stable cartilage is formed by the cell populations described by Quarto et al. it can not be alleged that Quarto et al. describes a method for the identification of positive (or negative) markers of chondrocyte (phenotypic) stability. Similarly, it is clear from the analysis of Quarto et al. that, independent of the method that is attributed to Quarto et al., it does not include the step of "identifying positive [or negative] molecular markers of the isolated or expanded cells which formed stable, non-vascularized cartilage in vivo, as evaluated in step e)" as presently claimed.

Applicants further note that where Quarto et al. considers *in vivo* differentiation potential (page 4971, section entitled "in vivo differentiation potential:

ectopic tissue formation in nude mice), the expression of collagen proteins by the cell populations used for injection in nude mice is not considered. In this regard, the *in vivo* experiments of Quarto et al. can also not be considered to comprise the step of 'identifying a positive [or a negative] marker *of those isolated or expanded cells which formed stable, non-vascularized cells in vivo*" as presently claimed (emphasis added). Quarto et al. in no way recognize or acknowledge the relevance of the expression of the collagen proteins in the ability for the cell population to make stable hyaline cartilage, let alone suggest that these proteins should be used for the identification of cell population with this phenotype.

As it is precisely an object of the present invention to identify those cells having the capacity to form stable, non-vascularized cartilage *in vivo* and that this is achieved by linking this capacity to the expression of positive and/or negative markers, it is clear that Quarto et al. do not anticipate the methods of the invention.

The Examiner has also rejected claim 36, relating to the sorting of phenotypically stable chondrocytes. The Examiner has stated that Quarto et al. teach that positive and negative markers can be detected using antibodies against those markers. Applicants note that, apart from the differences in methods, markers and chondrocyte stability discussed above, no mention or suggestion is made of sorting cells, let alone sorting phenotypically stable chondrocytes using antibodies. Applicants therefore submit that Quarto et al. fail to anticipate claim 36.

2. Claim Rejections under 35 U.S.C. § 103(a)

a) Claims 31-36, 43-45, 51, and 55 stand rejected under 35 U.S.C. § 103(a) as obvious over Quarto et al. in view of Kolettas et al.

As noted above above, Quarto et al. does not relate to chondrocyte (phenotypic) stability, i.e. the ability to form stable hyaline cartilage when injected *in vivo*.

In addition, as indicated above, Quarto et al., at most, relate to external factors affecting differentiation in the chondrogenic development pathway. In this context, Quarto et al. has evaluated the expression of collagen type I, II and X, as these are believed to be indicative of different stages of chondrocyte development (page 4966, right column, lines 13-25). There is no indication that the expression of these proteins is in anyway indicative of the ability to produce stable cartilage *in vivo*. To the contrary, it is noted that the expression of these proteins *per se* is not considered to be representative of the ability of cells to produce an organized extracellular matrix, relevant to chondrocyte differentiation. Indeed, Quarto et al. states: "The various types of collagen proteins synthesized by the cells, although properly secreted as shown in Fig. 3, could not be organized in either of these conditions." (page 4970, last paragraph). Thus, at most, Quarto et al. demonstrates that the assessment of collagen protein expression in vitro is not indicative of the ability to produce stable cartilage. Again, as indicated above, in view of the results provided by Quarto et al., applicants submit that Quarto et al. do not identify any conditions or any protein, the expression of which is clearly linked to the ability of the cells to form stable hyaline cartilage.

Kolettas et al. do not remedy the deficiencies of Quarto et al.. Kolettas et al. refer to markers "characteristic of cartilage" (see abstract, left column, lines 12-14), and as such compares the proteins expressed by different cell populations with those found in cartilage. Applicants note that this is not the same as markers for chondrocyte stability, defined as: "the capacity of a cell suspension, population of cell culture (either obtained from cartilage tissue or from any other tissue containing cells with chondrogenic potential) to produce upon injection in a mammal (in vivo), such as immune-deficient mice, (in a time-frame of three weeks) a cartilage implant without signs of vascular invasion or endochondral bone formation."

It is submitted that a marker expressed by cartilage, or a marker of the 'chondrocyte phenotype' as referred to by Kolettas et al. is not necessarily a marker of chondrocyte stability. Indeed, Kolettas et al. consider type X collagen as a positive marker of cartilage, which, according to the present invention, is a negative marker of chondrocyte stability.

In this regard it is noted that Kolettas et al. do not perform tests involving generation of cartilage tissue *in vivo*. Indeed, the analysis of the 'expression of the human chondrocyte phenotype' is performed by Kolettas et al. by determining morphology of the cells, growth characteristics in vitro, expression of cartilage collagens, proteoglycans and link protein (Kolettas et al. – see entire results section). Thus, it is submitted that there is no indication in Kolettas et al. that the markers suggested are of use in predicting the ability of cells to produce cartilage *in vivo*.

Moreover, with regard to the use of negative markers, it is noted that Kolettas et al. in fact doubt the existence of negative markers for the 'chondrocyte phenotype'. Indeed, Kolettas et al. state: "It is proposed that loss of the chondrocyte phenotype is marked by the loss of one or more cartilage-specific molecules rather than by appearance of non-cartilage-specific molecules." (abstract, last sentence). This indicates that Kolettas does not envisage negative markers, i.e. molecules, the absence of expression of which are indicative of the alleged 'chondrocyte phenotype', let alone negative markers of chondrocyte stability, as presently claimed.

Again, the Examiner has interpreted Kolettas et al. to suggest that collagen type X is used as a negative marker. However, it should be noted that this is not taught by Kolettas et al. Indeed Kolettas indicate that the cells are generally analysed for markers 'characteristic of cartilage'. The use of collagen type X is supported in this regard by a reference to Bonaventure et al (1994) which describes collagen type X as a marker expressed in cartilage. While Kolettas et al. note that there was no hybridisation to human collagen type X (page 1995, left column, lines 3-6) in the experiments performed, this result is not discussed and there is no indication that based thereon, this marker is in any way indicative of the ability of the cells to produce stable cartilage *in vivo*.

Moreover the Examiner has indicated that Quarto et al. point out that chondrocytes can be biopsied, cultured and expanded, then injected or grafted into an animal or patient, and the resulting cartilaginous tissue analyzed for markers indicating chondrocyte (phenotypic) stability. Applicants submit that this is

incorrect. As indicated, Quarto et al. does not analyze the resulting cartilaginous tissue obtained upon injection of cultured chondrocytes for markers. Indeed, as indicated above, in the *in vivo* experiments carried out by Quarto et al., no analysis of protein expression is performed. Nevertheless, even if this had been the case, Applicants submit that the methods of the present invention do not relate to the analysis of markers of the resulting cartilage but rather to the analysis of markers expressed by cell population prior to their implantation, thereby determining which markers correlate with the production of stable hyaline cartilage in vivo. This is in no way suggested by either of the cited references - Quarto et al. and Kolettas et al.

In view of the above, Applicants submit that the obviousness rejection in view of the combination of Quarto et al. and Kolettas et al. is inappropriate.

b) Rejection of claims 56 and 58 in view of the combination of Quarto et al., Kolleta et al., and Si et. al.

Claims 56 and 58 relate to the use of BMP-2 as a positive marker of chondrocyte stability.

As indicated above, it is contested that the methods for identifying a cell population having chondrocyte (phenotypic) stability are obvious in view of Quarto et al. and Kolettas et al. This is all the more true with regard to the use of BMP-2 in the methods of the invention as neither Quarto et al. nor Kolettas et al. describe the analysis of the expression of BMP-2 by chondrocytes, let alone suggest the use thereof as a marker for chondrocyte stability.

Si et al. teach that BMP-2 expression is not only correlated with the differentiation of mesenchymal cells into chondrocytes, but also with the differentiation into osteoblasts. As a requirement of chondrocyte stability implies the absence of bone formation, it is submitted that the observations by Si et al. does no suggest that BMP-2 expression is indicative of stable hyaline cartilage formation. Indeed, Si et al. state that "BMP may play an important role in bone induction". In addition, as detailed by Kolettas and Quarto et al. a marker for chondrocyte differentiation (e.g. collagen type X) is not necessarily a marker for the ability of chondrocytes to make stable hyaline cartilage *in vivo*.

Based on the above, no particular guidance or motivation is given to the skilled person, with regard to the use of BMP-2 as a marker for the ability of an isolated cell population to produce stable cartilage *in vivo*.

For these reasons, it is submitted that the obviousness rejection is inappropriate.

c) Rejection of claims 57 and 59 based on the combination of Quarto et al., Kolettas et al., Si et al., and Hamada et al.

Claims 57 and 59 relate to the specific use of FGFR-3 as a positive marker for the identification of cells with chondrocyte stability.

As detailed above, the combination of Quarto et al., Kolettas et al., and Si et al. in no way suggests the use of markers to identify cell populations capable of producing stable hyaline cartilage in vivo, let alone that they in any way disclose which markers would be appropriate for this purpose.

Hamada et al. describes the immunolocalization of the expression of fibroblast growth factor receptors (FGFRs) in rat mandibular condylar cartilage and tibial cartilage tissue, during different stages of expression. Differential expression of the different FGFRs in the different stages of development of cartilage is observed. It is concluded that the FGFRs play an important role in the differential growth pattern of the condylar cartilage.

Hamada et al. do not teach that FGFRs, let alone FGFR3 is expressed by isolated chondrocyte cell populations capable, when injected in vivo, of producing stable hyaline cartilage. Hamada does not discuss the production of cartilage by isolated cell populations. Hamada at most considers different stages of differentiation and markers indicative thereof in intact isolated tissue.

Applicants again note that the mere demonstration of the expression of FGFR-3 in cartilage or by cells obtained from cartilage tissue does not in itself teach the skilled person that this marker is indicative of the ability of an isolated cell population of producing stable hyaline cartilage in vivo. Indeed, Hamada also refers to the fact that this marker is also expressed in other cells, such as osteoblasts, osteocytes and fibroblasts (page 279 of Hamada et al., three first lines of the left column), cells which are definitely not capable of producing hyaline cartilage.

In view of the fact that Quarto et al. does not disclose or suggest a method for identifying cell populations capable of producing stable hyaline cartilage, nor discloses a method for identifying markers useful therein, that Kolettas similarly only addresses potential markers of differentiation and does not consider cartilage-

forming ability of isolated cell populations, that Si et al provide a marker which is characteristic of both osteoblast and chondrocyte development and that Hamada et al. provide a class of proteins of which only the involvement in differentiation is suggested, without any indication as to the relevance for cartilage formation of an isolated cell population in vivo, it is submitted that the prior art cited in no way describe or suggest the claims of the present invention.

CONCLUSION

Applicants submit that the application is in condition for allowance, and such action is respectfully requested.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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